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Setting the Scene

As mentioned in the previous chapter, flow cytometry has been moving in two directions at once. The earliest flow cytometers were either homemade Rube Goldberg (Heath Robinson, U.K.) monsters or, a few years later, equally complex, unwieldy commercial instruments. These were expensive; they were also unstable and therefore difficult to operate and maintain. For these reasons, the cytometer tended to collect around itself the trappings of what might be called a flow facility—that is, a group of scientists, technicians, students, and administrators, as well as a collection of computers and printers, that all revolved around the flow cytometer at the hub. If a scientist or clinician wanted the use of a flow cytometer to provide some required information, he or she would come to the flow facility, discuss the experimental requirements, make a booking, and then return with the prepared samples at the allotted time. The samples would then be run through the cytometer by a dedicated and knowledgeable operator. Finally, depending on the operator's assessment of the skill of the end-user, a number, a computer print-out, or a computer disk would be handed over for analysis.

Just at the moment when such flow facilities were beginning to see themselves as essential components of modern research, the technology of flow cytometry began to move in a new direction. Users and manufacturers both began to realize that different laboratories have different instrumentation needs. A sophisticated sorter might be necessary for certain applications, but its demands on daily alignment and skilled maintenance are time-consuming and its research capabilities might be superfluous for routine processing of, for example, clinical samples. Instead of simply continuing to become larger, more expensive, more complicated, and more powerful, some flow

cytometers started to become, in the late 1980s, smaller, less expensive, more accessible, and, although less flexible, remarkably stable. These instruments are called “benchtop” cytometers because they are self-contained and can be taken from their shipping crates, placed on a lab bench, plugged in, and (with luck) are ready to go.

The question arises as to how much training is required for use of these benchtop instruments. Pushing the buttons has become easy, but my prejudice on this issue should, of course, be obvious. If I believed that flow experiments could be designed and flow data could be acquired and analyzed appropriately by people with no awareness of the limitations and assumptions inherent in the technique, I would not have written this book. The actual operation of these small cytometers has been vastly simplified compared with that of the original research instruments. It certainly can be said that the new wave of cytometers has made flow analysis a great deal more accessible to the nonspecialist. A serious concern, however, is that the superficial simplicity of the instruments may lull users into a false sense of security about the ease of interpretation of the results. The basis for this concern is particularly clear in the medical community, where clinicians have been conditioned to expect that laboratory reports will contain unambiguous numbers; they may not be accustomed to the need for an intellectual framework in which to interpret those numbers. Anyone designing flow experiments or interpreting flow data needs some essential training in the technique; more training is required as the benchtop instruments (and the possible experiments) become more complex. At the high end, the operation of sorting instruments is best left to a dedicated operator; but, even here, the grass-roots user needs to understand flow cytometry in order to design an effective sorting protocol and communicate this protocol to the operator.

Although benchtop cytometers are less expensive than state-of-the-art instrumentation, they are still expensive. Therefore core facilities with shared instrumentation still provide for much of the current flow cytometric analysis. These shared facilities reflect a need by many for flow cytometric instrumentation, but also recognition of its high cost, its requirement for skilled maintenance and operation, and the fact that many users from many departments may each require less than full-time access. Such centralized facilities may have more than one cytometer. The trend now is to have one or more sophisticated instruments for specialized procedures accompanied by several bench-

top cytometers as routine work horses. There are usually dedicated operators who run the sophisticated instruments, supervise use of the benchtop instruments, and ensure that all instruments are well-tuned for optimum performance. In addition, there may be a network of computers so that flow data can be analyzed and re-analyzed at leisure—away from the cytometer and possibly in the scientist's own laboratory. A cytometry facility may also provide centrifuges, cell incubators, and fluorescence microscopes to aid in cell preparation. Increasingly, such institutional flow facilities are also becoming general cytometry facilities; the use of imaging microscopes for cell analysis has proved to be a technique that complements flow work. Similar stains are used in both flow and image analysis systems. Flow cytometry is based on analysis of light from a large number of cells as they individually pass a detector. Image analysis studies the distribution of light signals emanating from a large number of individual positions scanned over a single stationary cell.

The funding of such central facilities often involves a combination of institutional support, direct research grant support, and charges to users (or patients). The charges to users may vary from nominal to prohibitive and may need to support everything from consumables like computer paper and buffers to the major costs of laser replacement, service contracts, and staff salaries. With the increasing complexity and variety of instrumentation and with the need for data organization, booking systems, and financial accounting, the running of these flow facilities can become an administrative task in itself. Therefore a “corporate identity” often evolves, with logos designed, meetings organized, newsletters written, and training sessions provided.

Moreover, a funny thing has been happening. What originally appeared to be a rift in the field of flow cytometry, isolating the “high tech” from the “benchtop” users, has turned out to be, instead, a continuum. State-of-the-art instruments continue to improve, marking the advancing edge of technological and methodological development. However, benchtop flow cytometers have followed along behind, becoming increasingly powerful and combining the advantages of stable optics and fluidics with the new capabilities demonstrated on last year's research instruments.

In this book, emphasis will be on the general principles of cytometry that apply equally to benchtop and state-of-the-art instru-

mentation. The chapter on sorting will, however, provide some insight into the ability of research cytometers to separate cells. In addition, the chapter on research frontiers provides a glimpse into the advanced capabilities of today's research instruments (and, without doubt, many of these capabilities will soon appear on tomorrow's benchtop cytometers).